Amino acid availability in ruminants of cereals and cereal co-products

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Abstract

BACKGROUND: Microbial corrected *in situ* estimates of the ruminal undegraded fraction (RU) and intestinal effective digestibility (IED) of amino acids (AA), except tryptophan, of rye, wheat and corn grains, wheat bran, wheat and barley distilled dried grains and corn gluten feed were measured on three rumen- and duodenum-cannulated wethers using ^{15}N-labelling techniques and considering ruminal rates of particle comminution and outflow.

RESULTS: The lack of microbial correction led to overestimations of the intestinal digested fraction that rose with the increase in ruminal degradability. Thus these overestimations varied widely among feeds (from 4.3 to 32.1% for total analysed AA) and among AA. Digestion led to large changes in the AA supply that were greater in the rumen than in the intestine. The impact of these changes on the protein value is conditioned by the magnitude of the undegraded protein fraction.

CONCLUSION: Microbial contamination taking place in the rumen and changes in the AA supply with digestion should be considered to attain accurate estimates of AA digestion. Globally, digestion improved the AA supply in rye, wheat and wheat distilled dried grain and decreased it in corn and corn gluten feed by reducing the supply of valine and basic AA, especially lysine.

Keywords: ruminal degradability; intestinal digestibility; microbial contamination; amino acids; sheep

INTRODUCTION

Accurate estimates of the amino acids (AA) absorbed from feeds by ruminants are necessary to improve the precision of actual rationing systems and therefore nitrogen efficiency in these species. Owing to the extreme difficulties in attaining this evaluation through *in vivo* studies, it is currently tackled through *in situ* trials. However, the methodologies used in many of these assays have limitations biasing the results for the ruminal undegraded fraction and its intestinal digestibility. Thus AA availability studies have usually been performed using ruminal undegraded residues obtained at fixed incubation times, which do not represent the true ruminal residence time of feed particles.[1,2] Therefore the resultant values do not consider either the undegraded AA flowing before this fixed time or those flowing after it due to subsequent ruminal protein degradation. In addition, the feed rumen residence has usually been under-evaluated by considering only the rate of particle outflow from the rumen (k_p) and not the time used in the process of particle conditioning necessary for this escape, which is represented by the particle comminution rate (k_c).[2–4] Finally, in many of these studies the microbial contamination of feed particles in the rumen was not corrected in ruminal and intestinal estimates, in spite of the fact that this contamination may be important.[3–5]

Although the supply of intestinal digestible AA from cereal grains may be limited by its high protein degradability, as in the case of most grains of the Triticeae tribe, or by its low protein content, as in the case of corn, its net contribution may be important, because these feeds represent a large proportion of the diets used in high-productive ruminants. This contribution may increase in cereal co-products owing to its higher protein content and/or a reduction in its degradability associated with the industrial processing of these co-products. The aim of this work was to study, without the above indicated limitations, the effects of digestion on the availability of AA in seven samples of cereal grains and cereal co-products.

EXPERIMENTAL

Tested feeds, animals and feeding

Seven samples of cereal grains (rye (RG), wheat (WG) and corn (CG)) and cereal co-products (wheat bran (WB), distilled dried grains with solubles from wheat (DDGW) and barley (DDGB) and corn gluten feed (CGF)) were tested on three wethers fitted with rumen cannulae and T-type duodenal cannulae. Wethers were fed with a 2:1 (w/w) chopped oat hay/concentrate diet starting 10 days before the experimental period. Additional details on the animals and diets have been published previously.[3,4] The protein and AA compositions of tested feeds are given in Table 1.

Experimental procedures

These studies included two incubations of feeds in nylon bags in the rumen together with a transit study of the dietary concentrate particles in this compartment.[3] Kinetics of dry matter (DM) degradation were establish by fitting to single-exponential curves the DM disappearance recorded at times of 0 (non-washout fraction), 2, 4, 8, 16, 24 and 48 h. Then the ruminal undegraded fraction (RU) of these feeds was determined in each wether from both these studies as indicated in Arroyo and González,[2] considering both k_c and k_p rates. To correct for particle contamination with adherent microorganisms, a solution of ^{15}N-enriched ammonium sulfate was continuously infused into the rumen during this experimental phase and samples of solid adherent bacteria (SAB) were isolated and used as reference for these corrections. The SAB composition has been reported previously;[6] nonetheless, the protein and AA composition data are shown again in Table 1 to facilitate comparison with the data of tested feeds. Then, after a 10 day resting period to eliminate the ^{15}N enrichment in the digesta, a study of the intestinal digestion of the ruminal undegraded fraction (RU) of these feeds was performed using mobile nylon bags that were introduced through the duodenal cannula and recovered in the faeces. All details on these procedures have been published previously.[3,4] Effective estimates for AA of both RU and its intestinal digestibility (IED) were obtained from composite samples representative of the total rumen outflow of undegraded feed as proposed by Arroyo and González.[2] Thus the freeze-dried residues from both incubations were pooled in equal quantities for each animal and each incubation time. Then these last samples were mixed for each wether in proportions determined by the function describing the rumen outflow of undegraded feed considering both the k_c and k_p rates. For this purpose the residues at 0, 2, 4, 8, 16, 24 and 48 h of incubation were considered representative of the chemical composition of the feed rumen outflow up to times of 1, 3, 6, 12, 20, 36 and 60 h respectively and the above indicated proportions were calculated by the quotient of the flow in each interval and the total flow.

The RU of individual AA and TAA was determined from the RU of DM and their concentrations in the composite samples (Y) and in the whole feed (X) as

$$\text{RU} = Y \times \text{RU of DM}/X$$

The IED of individual AA and TAA was determined from the IED of DM and their concentrations in the composite samples (Y) and in the intestinal incubated residues (Z) as

$$\text{IED} = 1 - \left[Z \times \left(1 - \text{IED of DM}\right)/Y\right]$$

The microbial proportion of AA in composite samples and intestinal undigested residues was determined as the microbial DM content of these samples × the AA content (on DM) in SAB.

Chemical and statistical analyses

Analyses of AA in tested samples, incubated residues or SAB were conducted after acid hydrolysis using α-aminobutyric acid as internal standard. Therefore tryptophan was not measured in this study. This hydrolysis was preceded by an oxidation with performic acid to obtain methionine and cysteine values. These analyses were performed using a high-performance liquid chromatography (HPLC) system after derivatizing AA with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) in accordance with Liu *et al.*[7]

The effects of the microbial contamination correction (c) in RU, IED and the intestinal digested fraction (IDF) of TAA were studied by variance analyses by taking into account this primary factor as well as the wethers (w) in the model ($y_{ij} = \mu + c_i + w_j + \epsilon_{ij}$). Considering as primary factor the AA, this same design was also used to compare the microbial corrected results of individual AA *vs* TAA by protected least squares differences. Differences in relation to the AA profile of the intact feed of RU and IDF were analysed by t test of the differences between both. Effects of the factors were declared significant at $P < 0.05$ and trends were discussed at $P < 0.1$. All statistical analyses were performed using the GLM procedure of SAS.[8]

RESULTS

Apparent values of RU of DM were low in WG (84.4 g kg^{-1}) and RG (87.0 g kg^{-1}) and varied from 200 to 300 g kg^{-1} in the remaining feeds, whereas proportions of IED of DM ranged from 0.134 in WB to 0.694 in CG (Table 1). The lack of correction for microbial contamination in the rumen led to overestimations of TAA in RU, although this effect was recorded only as a tendency in WG and CG (Table 2). These overestimations were associated with others of moderate importance in the IED of TAA in CGF and as a tendency in RG (Table 2). As a consequence, the lack of this correction led also to overestimations in IDF, although in WG, WB and CG only the level of tendency was reached (Table 2). To reduce the extent of tables and to systematize the variations with this correction on digestive estimates of individual AA, only the range of these overestimations and the AA providing the extreme corrections are shown in Table 3. The range of RU overestimations among AA was large in most feeds and more moderate in distilled dried grains. Correlations between RU overestimations and the SAB/feed ratio of each AA were significant at $P < 0.001$ in all feeds (Table 3). The IED overestimation range was low or moderate except in CGF. In IDF this range was enlarged in relation to that recorded in RU except in distilled dried grains and WG. For both RU and IDF, proline displayed the minimum variations in most feeds, whereas maximum variations were recorded for tyrosine or lysine (Table 3).

Differences in individual AA in relation to TAA in microbial corrected values are shown in Tables 4–6, while variations in the AA profile of RU and IDF in relation to that of the intact feed are presented in Figs 1 and 2 for feeds of the Triticeae tribe and in Fig. 3 for CG and CGF. Globally, these results showed a large variability among feeds of digestion effects. For the usually considered most interesting AA in nutrition, digestion increased the lysine availability in RG and WG and decreased it in CG and CGF; also, digestion increased the availability of methionine in WB, DDGW and CGF as well as that of cysteine in DDGW and CGF.

Table 1. Protein content (g kg^{-1} of DM), amino acid profile (g kg^{-1} analysed amino acids) and apparent ruminal undegraded fraction (RU; g kg^{-1}) and its intestinal effective digestibility (IED; as proportion) of dry matter in cereal grains, cereal co-products and solid adherent bacteria of rumen content (SAB)

Component	RG[a]	WG	WB	DDGW	DDGB	CG	CGF	SAB
Crude protein	126	137	138	346	232	87.8	175	468
TAA[b]	95.1	101	131	295	217	72.5	142	427
Arginine	67.1	47.4	65.6	51.7	56.2	50.1	45.3	51.2
Histidine	20.7	25.6	34.4	27.0	29.4	27.5	41.4	19.1
Isoleucine	34.6	35.7	36.0	35.8	38.1	34.5	34.3	59.1
Leucine	67.4	69.9	69.1	71.7	74.0	119	101	77.6
Lysine	41.6	27.0	42.7	19.4	27.1	28.8	29.9	69.1
Metionine	14.0	15.9	17.2	16.0	18.5	20.8	18.4	19.8
Phenylalanine	51.7	45.6	44.3	48.0	56.1	46.3	36.5	71.4
Threonine	37.6	29.1	33.0	31.5	41.1	37.1	37.6	50.3
Valine	47.3	43.4	51.6	46.2	51.9	46.4	54.0	52.4
Alanine	43.5	35.6	50.2	40.0	45.6	81.8	88.5	56.5
Aspartic acid	64.7	54.0	77.6	55.1	64.3	78.9	69.5	123
Cysteine	19.2	20.8	21.3	19.4	21.4	19.2	26.7	21.2
Glutamic acid	264	342	244	310	238	207	189	129
Glycine	51.0	47.6	65.7	44.1	52.2	46.5	57.6	58.7
Proline	96.8	82.4	70.1	93.8	100	72.9	94.2	29.0
Serine	50.8	55.6	48.9	51.7	55.5	59.1	47.7	56.5
Tyrosine	27.9	22.3	28.4	38.2	30.8	24.5	28.3	56.3
EAA[c]	382	340	394	347	392	411	399	475
RU[d]	87.0	84.4	299	279	290	200	240	
	(3.62)	(2.44)	(10.0)	(20.4)	(22.5)	(15.2)	(13.0)	
IED[d]	0.214	0.266	0.134	0.448	0.208	0.694	0.141	
	(0.0161)	(0.0380)	(0.0115)	(0.0478)	(0.0041)	(0.0671)	(0.0240)	

[a] RG, rye grain; WG, wheat grain; WB, wheat bran; DDGW, distilled dried grains of wheat; DDGB, distilled dried grains of barley; CG, corn grain; CGF, corn gluten feed.
[b] Total analysed amino acids, tryptophan not included.
[c] Essential amino acids.
[d] Values in parentheses are standard deviations.

Table 2. Effects of correcting for the microbial contamination taking place in the rumen on *in situ* estimates of ruminal undegraded fraction (RU), intestinal effective digestibility (IED) and intestinal digested fraction (IDF) of total analysed amino acids in cereals and cereal co-products (values are expressed as proportions)

	RU				IED[a]				IDF			
Sample	NC[b]	C	SEM[c]	*P*	NC	C	SEM	*P*	NC	C	SEM	*P*
RG[d]	0.0797	0.0681	0.00153	0.033	0.0700	0.671	0.0052	0.057	0.0561	0.0460	0.0016	0.038
WG	0.0793	0.0702	0.00186	0.075	0.0815	0.808	0.0059	0.507	0.0650	0.0572	0.0018	0.088
WB	0.150	0.122	0.0045	0.046	0.696	0.641	0.0191	0.176	0.105	0.0795	0.0045	0.055
DDGW	0.312	0.299	0.0004	0.002	0.935	0.937	0.0003	0.044	0.292	0.280	0.0003	0.001
DDGB	0.184	0.169	0.0012	0.012	0.878	0.877	0.0018	0.763	0.162	0.149	0.0013	0.019
CG	0.290	0.255	0.0064	0.058	0.912	0.906	0.0037	0.326	0.265	0.232	0.0068	0.061
CGF	0.147	0.118	0.0001	< 0.001	0.759	0.719	0.0046	0.025	0.112	0.0855	0.0004	< 0.001

[a] Expressed on RU.
[b] NC and C, not corrected and corrected for microbial contamination respectively.
[c] SEM, standard error of mean; degrees of freedom (DF) = 2.
[d] RG, rye grain; WG, wheat grain; WB, wheat bran; DDGW, distilled dried grains of wheat; DDGB, distilled dried grains of barley; CG, corn grain; CGF, corn gluten feed.

DISCUSSION

Accuracy of *in situ* estimates

The AA concentrations of tested feeds were in the intervals defined by common nutritive value tables,[9–11] while SAB concentrations were normal for this bacterial population.[6]

The large variability among AA in the RU overestimation due to the lack of microbial correction may be associated with differences in AA contents between SAB and feeds as evidenced by the correlations shown in Table 3. Nevertheless, this effect is enlarged with the increase in the degradability of individual AA. Thus

Table 3. Range of overestimations (%) by not correcting for the microbial contamination taking place in the rumen of ruminal undegraded fraction (RU), intestinal effective digestibility (IED) and intestinal digested fraction (IDF) of amino acids of tested feeds

Feed	RU	r^a	IED	IDF
RG[b]	6.97 (Cys) – 46.0 (Tyr)	0.665	1.06 (Pro) – 14.1 (Cys)	8.93 (Pro) – 53.8 (Tyr)
WG	4.47 (Pro) – 39.9 (Tyr)	0.562	0.05 (Pro) – 3.88 (Gly)	4.41 (Pro) – 39.6 (Tyr)
WB	9.23 (Pro) – 84.7 (Tyr)	0.514	2.41 (Arg) – 34.1 (Tyr)	12.0 (Pro) – 121 (Tyr)
DDGW	1.36 (Pro) – 13.2 (Lys)	0.754	−0.52 (Tyr) – 0.00 (Cys)	0.00 (Glu) – 13.4 (Lys)
DDGB	2.72 (Pro) – 22.6 (Tyr)	0.683	−0.32 (Glu) – 1.75 (Asp)	2.31 (Pro) – 22.2 (Lys)
CG	4.09 (Pro) – 45.1 (Lys)	0.557	0.00 (Pro) – 19.0 (Lys)	4.36 (Pro) – 57.6 (Lys)
CGF	6.77 (Pro) – 72.7 (Lys)	0.830	1.30 (Glu) – 144 (Lys)	8.40 (Pro) – 251 (Lys)

[a] Correlation coefficients between RU overestimation and SAB/feed ratio for each amino acid ($n = 17$; significance of all correlations, $P < 0.001$).
[b] RG, rye grain; WG, wheat grain; WB, wheat bran; DDGW, distilled dried grains of wheat; DDGB, distilled dried grains of barley; CG, corn grain; CGF, corn gluten feed.

Table 4. Differences between individual and total analysed amino acids (TAA)[a] in microbial corrected *in situ* estimates of ruminal undegraded fraction of cereals and cereal co-products (values are expressed as proportions)

AA	RG[b]	WG	WB	DDGW	DDGB	CG	CGF
TAA	0.0681	0.0702	0.122	0.299	0.169	0.255	0.118
Arg	0.0854†	0.0769	0.111	0.283	0.162	0.158***	0.0830**
His	0.0667	0.0651	0.0912†	0.263	0.116**	0.219	0.0993†
Ile	0.0786	0.0754	0.135	0.332	0.167	0.246	0.108
Leu	0.0733	0.0693	0.132	0.319	0.174	0.309*	0.136†
Lys	0.135***	0.110***	0.118	0.356**	0.185	0.195*	0.0915*
Met	0.0855†	0.0826	0.161*	0.359**	0.175	0.278	0.154**
Phe	0.0763	0.0676	0.121	0.302	0.118**	0.295†	0.143*
Thr	0.104***	0.0920*	0.133	0.331	0.168	0.213†	0.159***
Val	0.0832	0.0757	0.130	0.319	0.179	0.242	0.107
Ala	0.108***	0.0947**	0.139	0.284	0.177	0.278	0.0926*
Asp	0.116***	0.0969**	0.153†	0.276	0.178	0.190**	0.100†
Cys	0.0875*	0.0809	0.159*	0.416***	0.174	0.267	0.147**
Glu	0.0355**	0.0544†	0.0950	0.297	0.188	0.265	0.118
Gly	0.113***	0.0871*	0.143	0.278	0.148	0.194*	0.113
Pro	0.0534	0.0716	0.130	0.295	0.184	0.342***	0.133
Ser	0.0725	0.0685	0.143	0.295	0.150	0.223	0.132
Tyr	0.0643	0.0586	0.0693**	0.224**	0.124*	0.283	0.130
SEM[c]	0.0058	0.0065	0.0160	0.0119	0.0162	0.0131	0.0072

[a] Values in the same column with a superscript are different from the TAA value at
$^{\dagger}P < 0.1$,
$^{*}P < 0.05$,
$^{**}P < 0.01$ or
$^{***}P < 0.001$.
[b] RG, rye grain; WG, wheat grain; WB, wheat bran; DDGW, distilled dried grains of wheat; DDGB, distilled dried grains of barley; CG, corn grain; CGF, corn gluten feed.
[c] SEM, standard error of mean; DF = 34.

AA displaying minimum and maximum RU overestimations also displayed similar SAB/feed values except in RG and DDGB. In RG, proline did not provide the minimum overestimation because its high degradability enlarged this error, whereas lysine did not reach the maximum overestimation in DDGB for the opposite reason. The magnitude of the associated IED overestimation with the digestion in the intestine of the adherent microorganisms is mainly a function of the difference in intestinal disappearance between feed and SAB.[1,3,12] Thus the lack of effects on the IED of TAA in most feeds may be justified by the low magnitude of this difference. The combination of RU and IED errors enlarged these overestimations on IDF, which for TAA varied from 4.3% (DDGB) to 32.1% (WB). The high variability of IDF overestimations among AA within the same feed and their large magnitude usually reached show the need to correct this contamination in all tested feeds to obtain accurate values of intestinal digested AA, in particular in those with low protein concentration and high protein degradability.

Amino acid digestion and feed protein value

The results of this study should be considered with caution, since only one sample of each feedstuff was tested. The comparison of digestive estimates of individual AA *vs* TAA or of RU or IDF profiles with that of the original feed showed that digestion led to large changes in the AA supply and also that ruminal degradation had a greater influence than post-ruminal digestion in these changes.

Table 5. Differences between individual and total analysed amino acids (TAA)[a] in microbial corrected *in situ* estimates of intestinal effective digestibility of cereals and cereal co-products (values are expressed as proportions over ruminal undegraded fraction)

AA	RG[b]	WG	WB	DDGW	DDGB	CG	CGF
TAA	0.671	0.808	0.641	0.937	0.877	0.906	0.719
Arg	0.736	0.854*	0.788**	0.922†	0.911	0.899	0.702
His	0.732	0.854*	0.761*	0.906***	0.911	0.888	0.766
Ile	0.641	0.754*	0.596	0.937	0.855	0.888	0.721
Leu	0.621	0.759*	0.601	0.932	0.849	0.949	0.826*
Lys	0.741	0.783	0.569	0.877***	0.819*	0.668*	0.218***
Met	0.500***	0.785	0.780*	0.912**	0.837	0.891	0.778
Phe	0.684	0.794	0.611	0.945	0.811	0.923	0.756
Thr	0.663	0.788	0.587	0.923†	0.864	0.758*	0.586*
Val	0.656	0.781	0.626	0.939	0.869	0.893	0.745
Ala	0.630	0.750*	0.547†	0.894***	0.824†	0.937	0.771
Asp	0.633	0.725***	0.561	0.881***	0.799**	0.833	0.608*
Cys	0.298***	0.529***	0.414***	0.890***	0.738***	0.853	0.765
Glu	0.819**	0.918***	0.777*	0.971***	0.939*	0.945	0.848**
Gly	0.462***	0.644***	0.478**	0.842***	0.763***	0.765*	0.390***
Pro	0.754†	0.849†	0.703	0.962**	0.935*	0.938	0.704
Ser	0.612	0.751*	0.566	0.922†	0.818	0.862	0.584**
Tyr	0.730	0.859*	0.558	0.961**	0.917†	0.918	0.772
SEM[c]	0.0155	0.0309	0.0320	0.0371	0.0055	0.0187	0.0318

[a] Values in the same column with a superscript are different from the TAA value at
†$P < 0.1$,
*$P < 0.05$,
**$P < 0.01$ or
***$P < 0.001$.
[b] RG, rye grain; WG, wheat grain; WB, wheat bran; DDGW, distilled dried grains of wheat; DDGB, distilled dried grains of barley; CG, corn grain; CGF, corn gluten feed.
[c] SEM, standard error of mean; DF = 34.

Thus correlation coefficients between RU and IDF values (0.849, 0.897, 0.843, 0.987, 0.965, 0.988 and 0.891 in RG, WG, WB, DDGW, DDGB, CG and CGF respectively; $P < 0.001$ in all feeds) were higher than those between IED and IDF (0.433, 0.443, 0.650, 0.255, 0.497, 0.787 and 0.752 respectively; $P < 0.001$ except in RG and WG ($P < 0.01$) and DDGW ($P < 0.1$)).

The changes in AA proportions associated with ruminal fermentation are mandatorily linked to differences among feed proteins in both degradability and AA composition, although both factors are partially related by the hydropathy profile of proteins.[1] Thus soluble and easily degradable proteins are rich in polar and/or hydrophilic AA (acid and basic AA, threonine, tyrosine, glycine and serine), which are expected to be degraded to a higher extent than those included in insoluble proteins, which are rich in non-polar and hydrophobic AA (branched-chain AA, cystine, methionine and phenylalanine). Other factors such as protein location in the cell and feed tissues,[13] the accessibility of microbial enzymes to proteins and the distribution in time of the feed post-ruminal flow (see later) may also condition differences in ruminal fermentation among AA through the differences in protein degradation resistance.

Differences in IED among AA and also among feeds should be determined by the concentration of indigestible components in RU, which in turn is conditioned by their concentration in the feed and its passive enrichment promoted by the ruminal fermentation.[1–3] Thus IED results showed lower values in feeds with higher fibrous components (i.e. RG *vs* WG, WB *vs* WG and CGF *vs* CG). Also, IED values were lower with the extent of the ruminal degradation. As cell wall proteins should have a lower intestinal digestibility than storage or other feed proteins, this suggests a higher ruminal escape of cell wall proteins. Thus, although differences in protein composition should be scarce between WG and DDGW, IED values were lower in the grain, because its presumably higher proportion of cell wall proteins in RU derived from its higher degradation. Cell wall proteins (extensins, glycine-rich proteins, cysteine-rich thionins, etc.) are rich in some AA types,[14] which therefore may be poorly digested in the small intestine. Thus cysteine displayed the minimum IED values (largely lower than those of TAA) in all tested feeds of the Triticeae tribe except DDGW, in which this position was for glycine, which also displayed the second minimum values in all these feeds. Glycine IED values were also largely lower than those of TAA in CG and CGF. Cysteine and glycine also displayed the minimum IED values in other feeds, decreasing markedly their intestinal digestion in highly fibrous feeds such as lucerne hay,[1] dehydrated sugar beet pulp[3] or expeller palm kernel and rapeseed meal,[4] which suggests that this inefficiency is associated mainly with the intestinal digestion of cell wall proteins. Structural glycine-rich proteins have glycine contents up to 60–70% of all AA residues and are integrated in the xylem,[15] tissue that shows a high resistance to the ruminal degradation.[16] Therefore a sizable proportion of the glycine supply to the intestine may be from these proteins. A similar argument may be possible for cysteine, as thionins may have toxic effects on bacteria and fungi, reducing microbial accessibility, and present numerous di-sulfur bonds,[17] which give a strong hydrophobic character and high degradation resistance to this AA.[18] Both characteristics may reduce their degradability and therefore increase the proportion of cysteine

Table 6. Differences between individual and total analysed amino acids (TAA)[a] in microbial corrected *in situ* estimates of intestinal digested fraction of cereals and cereal co-products (values are expressed as proportions)

AA	RG[b]	WG	WB	DDGW	DDGB	CG	CGF
TAA	0.0460	0.0572	0.0795	0.280	0.149	0.232	0.0855
Arg	0.0637†	0.0661	0.0874	0.261	0.148	0.142***	0.0588*
His	0.0496	0.0560	0.0698	0.239†	0.106*	0.195	0.0765
Ile	0.0512	0.0583	0.0810	0.311	0.143	0.220	0.0796
Leu	0.0460	0.0534	0.0799	0.297	0.149	0.293*	0.113*
Lys	0.0999***	0.0875***	0.0684	0.313	0.153	0.144***	0.0243***
Met	0.0436	0.0649	0.126**	0.327*	0.146	0.218	0.120**
Phe	0.0530	0.0544	0.0762	0.286	0.0995*	0.273†	0.109*
Thr	0.0694*	0.0731†	0.0780	0.287	0.146	0.165**	0.0945
Val	0.0552	0.0595	0.0824	0.299	0.156	0.217	0.0799
Ala	0.0685*	0.0712†	0.0808	0.255	0.147	0.261	0.0715
Asp	0.0744**	0.0710†	0.0934	0.243	0.143	0.162**	0.0626*
Cys	0.0275†	0.0429†	0.0662	0.371***	0.129	0.229	0.115**
Glu	0.0292†	0.0503	0.0754	0.289	0.177	0.251	0.100
Gly	0.0528	0.0564	0.0688	0.234†	0.113†	0.151**	0.0456***
Pro	0.0403	0.0612	0.0946	0.284	0.173	0.321***	0.0941
Ser	0.0449	0.0521	0.0842	0.273	0.123	0.193†	0.0776
Tyr	0.0481	0.0507	0.0433*	0.215*	0.115†	0.261	0.102
SEM[c]	0.0057	0.0066	0.0160	0.0118	0.0162	0.0131	0.0072

[a] Values in the same column with a superscript are different from the TAA value at
$^{\dagger}P < 0.1$,
$^{*}P < 0.05$,
$^{**}P < 0.01$ or
$^{***}P < 0.001$.
[b] RG, rye grain; WG, wheat grain; WB, wheat bran; DDGW, distilled dried grains of wheat; DDGB, distilled dried grains of barley; CG, corn grain; CGF, corn gluten feed.
[c] SEM, standard error of mean; DF = 34.

from cysteine-rich thionins in the RU fraction of this AA. The minimum IED obtained for lysine in CG and CGF and the decrease in these values in CGF seem to indicate that a high proportion of the RU lysine should also be provided by cell wall proteins, in which it is abundant.[14] This fact is favoured by a high proportion of zeins (which lack lysine[19]) in the RU protein fraction (see later). Results of O'Mara *et al.*[20] on 12 h rumen-incubated residues of corn distilled dried grains with solubles and corn gluten feed also showed a lower intestinal digestibility of lysine than TAA in both co-products and that this reduction was lower in the former (9.9%) than in the latter feed (34.4%), which included bran.

The large variations in all feeds of the essential AA (plus cysteine) profile of IDF in relation to that of the intact feed as well as IDF differences between individual AA and TAA show that large errors are made when a constant availability of AA is considered and therefore the AA profile of the intact feed is assumed as that of the intestinal absorbed AA.

In RG and WG, digestion improves the availability of some essential AA such as lysine and threonine, whereas in CG it improves that of leucine and phenylalanine but decreases that of arginine, lysine, threonine and valine. This opposite behaviour for lysine should be associated with the degradation resistance of proteins and the time in which the flow of undegraded proteins occurs. Thus albumins and globulins, which are the major sources of lysine in cereals,[21] may represent a significant part of the RU fraction in Triticeae grains whose flow occurs mainly in the first few hours after ingestion as a consequence of its fast degradation.[5,22] This lysine enrichment is facilitated by the low degradation resistance of Triticeae prolamines because they are found in poorly separated particles.[23] Thus Fahmy *et al.*[24] showed a fast degradation of prolamins similar to that of albumins and globulins in wheat. On the contrary, the post-ruminal flow of CG occurs over a prolonged time owing to its slow degradation;[5,24] in these conditions, zeins lacking lysine should represent a large component of RU protein. Zeins are markedly more degradation-resistant than albumins and globulins[24,25] owing to their location in resistant protein bodies in the endosperm and their intra- and intermolecular disulfide crosslinkages (provided mainly by β- and γ-zeins[26]).

The large increase in lysine in IDF of RG and WG (>40%) as well as in other essential AA has however a limited impact on the protein value of these grains as a consequence of their reduced fraction of undegraded protein. There was a general similarity between WG and WB profiles, although in WB the lysine availability was not improved. The similarity between WG and DDGW profiles was lower than with WB. The inclusion of yeast in distilled dried grains or the insolubilization of proteins by heat in the industrial process may contribute to these differences. Results of Table 4 show that ruminal digestion improved the content of sulfur-containing AA in both wheat co-products, in RG and numerically in WG. This fact may be associated with a higher degradation resistance of sulfur-rich prolamins due to the abundance of di-sulfur bonds. These proteins represent 70–80% of the prolamin fraction in Triticeae species and are rich in both cysteine and methionine.[27] However, owing to the low IED of cysteine, this positive effect is only reflected in the IDF in DDGW. In WB the high increase in methionine largely compensated the effect of the low IED of cysteine, whereas in DDGW this increase was higher in cysteine than in methionine. Digestion had global positive effects on the DDGW protein value, because in addition it had no negative effects on lysine (a numerical increase higher than 10% was

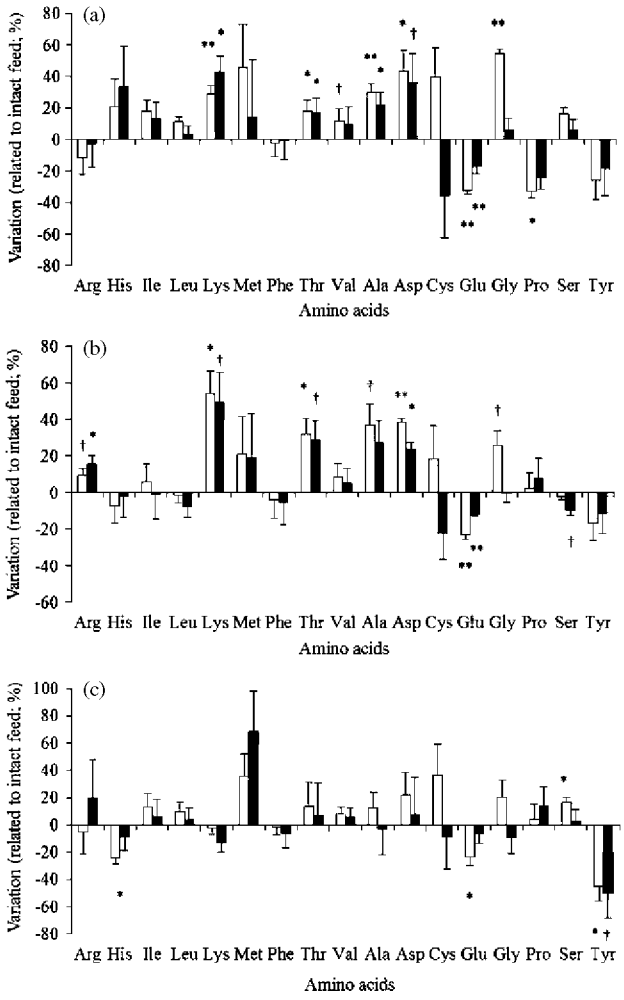

Figure 1. Variations (%) in amino acid profile of ruminal undegraded (RU, ■) and intestinal digested (IDF, □) fractions in relation to intact feed of (a) rye grain, (b) wheat grain and (c) wheat bran (values corrected for microbial contamination and estimated considering particle comminution and outflow rates). Bars are standard error of difference (SED); $^{\dagger}P < 0.1$; $^{*}P < 0.05$; $^{**}P < 0.01$.

recorded) and improved the availability of branched-chain AA. Digestion benefits on the AA supply were however reduced in WB by the limited values of ruminal escape and IED of its protein, whereas these benefits were enhanced in DDGW for the opposite reasons. Digestion had sparse effects on the essential AA profile of DDGB except for an impoverishment in histidine and phenylalanine.

Effects of ruminal digestion in CG agree with the above indication that zeins should represent a large fraction of the RU protein. Thus zeins, in particular α-zeins (which represent about 70% of the prolamins of this grain), lack lysine and are poor in arginine and, at a lower level, in histidine, glycine and aspartic acid, whereas they are extremely rich in proline, leucine and alanine.[26] There was also a general similarity between CG and CGF profiles except for a better use of threonine and cysteine in CGF. In this last feed, digestion increased the availability of phenylalanine and sulfur-containing AA but decreased that of basic AA (arginine, histidine and especially lysine) and valine. The large reduction in lysine availability aggravates the natural deficiency in CG, CGF and probably in other co-products including a significant fraction of corn bran or rich in zeins.

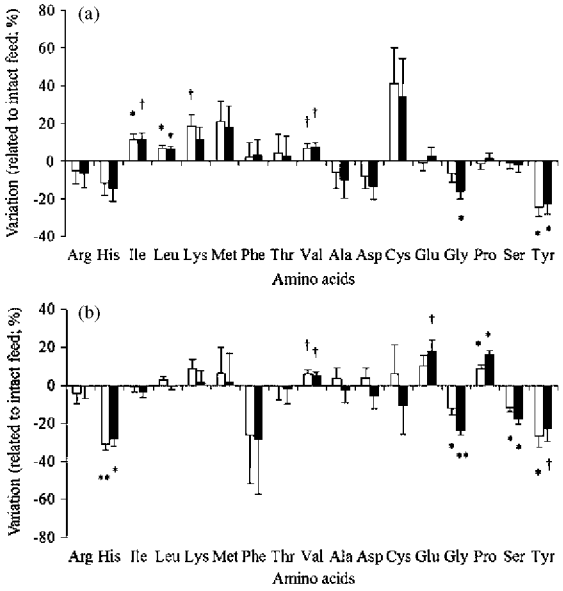

Figure 2. Variations (%) in amino acid profile of ruminal undegraded (RU, ■) and intestinal digested (IDF, □) fractions in relation to intact feed of distilled dried grains with solubles from (a) wheat and (b) barley (values corrected for microbial contamination and estimated considering particle comminution and outflow rates). Bars are SED; $^{\dagger}P < 0.1$; $^{*}P < 0.05$; $^{**}P < 0.01$.

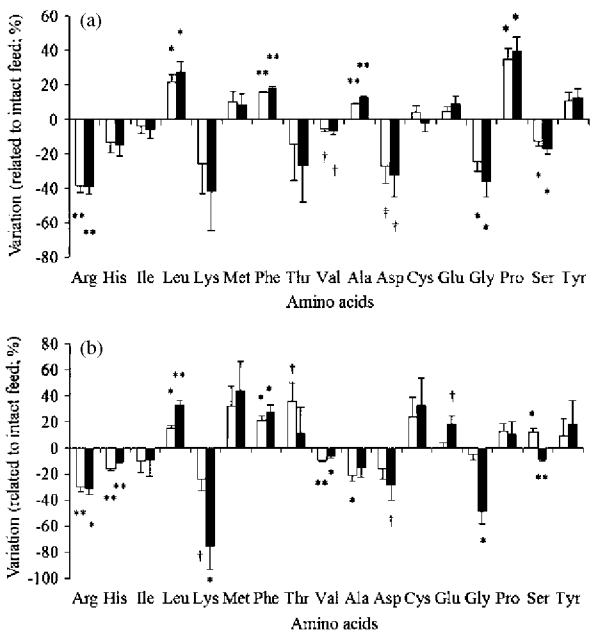

Figure 3. Variations (%) in amino acid profile of ruminal undegraded (RU, ■) and intestinal digested protein (IDF, □) fractions in relation to intact feed of (a) corn grain and (b) corn gluten feed (values corrected for microbial contamination and estimated considering particle comminution and outflow rates). Bars are SED; $^{\dagger}P < 0.1$; $^{*}P < 0.05$; $^{**}P < 0.01$.

CONCLUSIONS

Effects of the microbial contamination taking place in the rumen are largely variable among feeds and among AA and enlarge with the increase in their degradability. This contamination should be corrected to attain accurate estimates of ruminal and intestinal digestion of AA in these feeds. Digestion, especially in the rumen, led to considerable changes in the supply of essential AA and

cysteine to the animal. Ruminal changes are associated with differences in both the degradation resistance among proteins and the distribution in time of the post-ruminal flow of undegraded feed. Changes by intestinal digestion seem to be mainly associated with a low digestibility of cell wall proteins. Globally, digestion improved this supply in RG, WG and DDGW, whereas in CG and CGF it improved the supply of leucine and phenylalanine but reduced the supply of valine and basic AA, especially lysine as a consequence of its low escape and intestinal digestibility.

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